# PATENT COOPERATION TREATY REC'D 0 7 FEB 2006

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### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference FOR FURTHER AC		TION	See Form PCT/IPEA/416		
DELBE/P32303PC					
International application No. PCT/GB2004/005462	International filing date (d 23.12.2004	lay/month/year)	Priority date (day/month/year) 23.12.2003		
International Patent Classification (IPC) or national classification and IPC					
C12N15/80, C12N15/67, C12N5/10			·		
Applicant					
DELTA BIOTECHNOLOGY LIMITE	ED et al.				
<ol> <li>This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</li> </ol>					
2. This REPORT consists of a total	2. This REPORT consists of a total of 6 sheets, including this cover sheet.				
•	3. This report is also accompanied by ANNEXES, comprising:				
a. $oxtimes$ sent to the applicant and to the International Bureau) a total of 1 sheets, as follows:					
sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).					
☐ sheets which superse	de earlier sheets, but wh	ich this Authority consi	ders contain an amendment that goes		
beyond the disclosure Supplemental Box.	in the international appl	ication as filed, as indic	cated in item 4 of Box No. I and the		
b. (sent to the International E	B <i>ureau only)</i> a total of (in	dicate type and numbe amouter readable form	r of electronic carrier(s)) , containing a only, as indicated in the Supplemental		
Box Relating to Sequence	Listing (see Section 802	2 of the Administrative	Instructions).		
4. This report contains indications re	elating to the following ite	ems:			
☐ Box No. I Basis of the op	inion				
☐ Box No. II Priority					
☐ Box No. III Non-establishn	nent of opinion with rega	rd to novelty, inventive	step and industrial applicability		
☐ Box No. IV Lack of unity of					
Box No. V Reasoned state applicability; cit	ement under Article 35(2 tations and explanations	) with regard to novelty supporting such stater	r, inventive step or industrial ment		
☐ Box No. VI Certain docum	ents cited				
☐ Box No. VII Certain defects	s in the international appl	ication			
☐ Box No. VIII Certain observ	ations on the internation	al application			
Date of submission of the demand		Date of completion of th	is report		
19.07.2005		07.02.2006			
ranto and making address of the intervention		Authorized Officer	ches Patentan.		
preliminary examining authority:  ———————————————————————————————————	3. 5818 Patentlaan 2		The state of the s		
NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl		Aslund, J	Synau O)) an Paron		
Fax: +31 70 340 - 2040 1X. 3		Telephone No. +31 70 3	340-4393		

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/GB2004/005462

	Box No. I	Basis of the report	
١.	With regard	d to the <b>language</b> , this report is based on the international application in the language in which it was s otherwise indicated under this item.	
	which I inte	eport is based on translations from the original language into the following language, is the language of a translation furnished for the purposes of: ernational search (under Rules 12.3 and 23.1(b)) colication of the international application (under Rule 12.4) ernational preliminary examination (under Rules 55.2 and/or 55.3)	
2.	have been	d to the <b>elements</b> * of the international application, this report is based on <i>(replacement sheets whic</i> furnished to the receiving Office in response to an invitation under Article 14 are referred to in this originally filed" and are not annexed to this report):	
	Description	ı, Pages	
	1-130	as originally filed	
Sequence listings part of the description, Pages			
	1-27	as originally filed	
	Claims, Nu	ımbers	
	5-75	as originally filed	
	1-4	filed with telefax on 06.01.2006	
	Drawings,	Sheets	
	1/63-63/63	as originally filed	
	⊠ a sec	uence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing	
3.	□ th □ th □ th □ th	e description, pages e claims, Nos. e drawings, sheets/figs e sequence listing (specify): ny table(s) related to sequence listing (specify):	
4	had not b Suppleme  th  th  th  th	report has been established as if (some of) the amendments annexed to this report and listed below een made, since they have been considered to go beyond the disclosure as filed, as indicated in the ental Box (Rule 70.2(c)).  The description, pages the claims, Nos.  The drawings, sheets/figs  The drawings, sheets/figs  The sequence listing (specify):  The provided History (specify):  The provided History (specify):	
	* If i	tem 4 applies, some or all of these sheets may be marked "superseded."	

#### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

No:

Yes: Claims

Claims

1-75

Inventive step (IS)

Yes: Claims

1-75

Claims No:

Industrial applicability (IA)

Yes: Claims

1-75

Claims No:

2. Citations and explanations (Rule 70.7):

see separate sheet

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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	Supple	emental Box relating to Sequence Listing			
Continuation of Box I, item 2:					
1.	With re	h regard to any <b>nucleotide and/or amino acid sequence</b> disclosed in the international application and sessary to the claimed invention, this report has been established on the basis of:			
	a. type of material:				
	$\boxtimes$	a sequence listing			
		table(s) related to the sequence listing			
b. format of material:					
	$\boxtimes$	in written format			
	$\boxtimes$	in computer readable form			
	c. time	of filing/furnishing:			
	$\boxtimes$	contained in the international application as filed			
	$\boxtimes$	filed together with the international application in computer readable form			
		furnished subsequently to this Authority for the purposes of search and/or examination			
		received by this Authority as an amendment on			
2.	th a	addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating ereto has been filed or furnished, the required statements that the information in the subsequent or ditional copies is identical to that in the application as filed or does not go beyond the application as filed, appropriate, were furnished.			

3. Additional observations, if necessary:

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#### Re Item V.

Reference is made to the following documents:

- D1: MARTZEN MARK R ET AL: "A biochemical genomics approach for identifying genes by the activity of their products" SCIENCE (WASHINGTON D C), vol. 286, no. 5442, 5 November 1999 (1999-11-05), pages 1153-1155, XP002325596 ISSN: 0036-8075
- D2: "pYEX4T-1 Vector Information" 1998, CLONTECH CATALOG #6196-1, XP002325601
- D3: PAREKH RAJESH N ET AL: "Expression level tuning for optimal heterologous protein secretion in Saccharomyces cerevisiae" BIOTECHNOLOGY PROGRESS, vol. 13, no. 2, 1997, pages 117-122, XP002325597 ISSN: 8756-7938
- D4: BAO W-G ET AL: "Secretion of human proteins from yeast: stimulation by duplication of polyubiquitin and protein disulfide isomerase genes in Kluyveromyces lactis" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 272, no. 1-2, 11 July 2001 (2001-07-11), pages 103-110, XP004274844 ISSN: 0378-1119

#### Inventive step - Article 33(3) PCT

The application concerns co-expression of a target protein and a chaperone from a 2-micron plasmid. The application states that a technical prejudice in the prior art with regard to expression of proteins from 2 micron plasmids has been overcome. The application cites (pages 3-5) documents such as D3, D4 which state that expression from 2-micron constructs is less efficient than from constructs integrated on the chromosome. Said documents speculate that this is due to overloading of the secretory machinery of the cell including overloading of chaperone functions of the secretory pathway. Regarding expression of cytosolic target proteins, D4 provides an example where expression of a ubiqutin from a 2-micron plasmid is toxic - an effect which is overcome by chromosomal integration of the construct. On the other hand D1 shows expression on a genomewide basis of proteins from a 2 micron plasmid (see D2). However, there is no teaching in the prior art that would prompt a person to attempt co-

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#### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

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expression of a chaperone and a target protein from a 2-micron plasmid. Should a person skilled in the art want to test the effect of co-expression of a chaperone along with a target protein, the approach would be conservative. I.e, in view of D3, D4, a person skilled in the art would be discouraged to include a reading frame for a chaperone on the same 2-micron plasmid as the target protein and instead opt the safer approach, namely chromosomal integration of the chaperone co-expression construct.

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#### **CLAIMS**

- 1. A method for producing non-2 µm-family plasmid protein comprising:
- 5 (a) providing a host cell comprising a 2µm-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2µm-family plasmid protein;
- (b) culturing the host cell in a culture medium under conditions that allow the ιο- expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2μm-family plasmid protein; and
- (c) purifying the thus expressed non-2µm-family plasmid protein from the cultured host cell or the culture medium.;
  - 2. The method of Claim 1 further comprising the step of formulating the purified non-2µm-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.
  - 3. Use of a 2µm-family plasmid as an expression vector to increase the production of a fungal (preferably yeast) or vertebrate non-2µm-family plasmid protein by providing a gene encoding the non-2µm-family plasmid protein and a gene encoding a chaperone protein on the same 2µm-family plasmid.
  - 4. A 2μm-family plasmid comprising a gene encoding a protein comprising the sequence of a chaperone protein and a gene encoding a non-2μm-family plasmid protein, wherein if the plasmid is based on the 2μm plasmid then it is a disintegration vector.